8-2006

Fat Stores, Plumage Morphs, and Sex of Migrant White-throated Sparrows

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Fat Stores, Plumage Morphs, and Sex of Migrant White-throated Sparrows.

A Thesis
Presented to the Graduate Faculty of the Department of Biological Sciences
Of the State University of New York College at Brockport
In Partial Fulfillment for the Degree of
Master of Science

By
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August 2006

THESIS DEFENSE

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APPROVED	NOT APPROVED	MASTER'S DEGREE ADVISORY COMMITTEE
✓		Major Advisor — Date: 8 Sept 06
✓		Committee Member — Date:
✓		Committee Member — Date: Sept 8, 2006

Chairman, Graduate Committee

Chairman, Dept. of Biological Sciences — 9/26/06

Abstract - A between-season comparison of lipid stores carried by migrant White-throated Sparrows, as determined by deuterium dilution and visible fat class.

I compared qualitative and quantitative measures of fat stores for 144 migrant White-throated Sparrows (*Zonotrichia albicolis*) in fall and spring. Qualitative measures were visible fat classes and a morphometric index (100 * mass / wind chord). I calculated lipid index (fat mass / fat-free dry mass) for the quantitative measure of fat stores using the deuterium dilution technique. Spring migrants had significantly higher fat class (2.0 ± 0.1 vs. 1.0 ± 0.2), lipid index (1.06 ± 0.03 vs. 0.46 ± 0.03), and condition index (36.71 ± 0.30 vs. 34.27 ± 0.24) than fall migrants. Spring migrants also had higher lipid index values for a given fat class than did fall migrants. Spring linear regression slopes of the relationship between fat class and lipid index were steeper than fall regression slopes. Since fat classes do not consistently estimate fat storage between seasons, I advise against comparing fat classes between different seasons. Differences in areas of fat deposition or fat consumption between seasons may account for seasonal differences in lipid index values for individual fat classes.

Abstract - Is wing chord a useful criterion to sex White-throated Sparrows?

Determining the sex of White-throated Sparrows (*Zonotrichia albicolis*) outside of the breeding season can be difficult. Males tend to be larger than females and therefore wing chord length has been used to sex some individuals. However, overlap in wing chord length between males and females means that some individuals may be incorrectly sexed, while the sex of other individuals cannot be determined using wing chord criteria. I determined the sex of 159 White-throated Sparrows using molecular techniques and then examined the distribution of wing chord lengths for both sexes. In this study, all individuals with wing chord lengths $\geq$ 73.5 mm were males and all individuals with wing chord lengths $\leq$ 66.5 mm were females. Using Pyle's (1997) wing chord length criteria for sexing White-throated Sparrows (females < 69 mm, males > 72 mm), only 3% of males and 1% of females were mis-identified; however, only 42% of males and 16% of females could be safely separated from the opposite sex based on Pyle's wing chord length criteria. These data suggest that studies of White-throated Sparrows, and perhaps many other passerine species that require accurate sexing of individuals during the non-breeding season, may necessitate the use of molecular sexing techniques.

Abstract - Does lipid index differ between plumage and sex classes of migrant White-throated Sparrows?

White-throated Sparrows (*Zonotrichia albicollis*) display a plumage and behavioral polymorphism that is genetically determined. Birds with white colored crown stripes are generally more aggressive, territorial, sing more, seek more extra-pair copulations and contribute less parental care toward offspring than birds with tan colored crown stripes. Negative assortative mating by the plumage morphs maintains this polymorphism. However, there is a higher observed frequency of white-striped (WS) male x tan-striped (TS) female pairs than TS male x WS female pairs on the breeding grounds. I proposed that differences in lipid reserves carried during fall and spring migrations might be related to the unequal frequencies of pair types observed on the breeding grounds. I looked for differences in lipid index between plumage and sex classes. In the fall, I found that TS females had lower lipid index than other plumage and sex classes for White-throated Sparrows captured at Braddock Bay on the southern shore of Lake Ontario. I also looked for evidence within each sex of unequal proportions of plumage classes in fall and spring migrants. Based on my data, TS females were significantly more common than WS females in the fall, but not in spring. Using wing chord criteria to determine sex, I also analyzed spring banding data between 1992 and 1999 from Braddock Bay Bird Observatory (Greece, NY) and found significantly more WS than TS males and significantly more TS than WS females. However, I also found that for each sex, WS birds had significantly larger wing chord lengths than TS birds. Thus, I question any within sex plumage morph frequency data that use wing chord to determine sex.

ACKNOWLEDGEMENTS

First, I want to give a big 'thank you' to Dr. Christopher Norment for advising me, reviewing my drafts, and reining me in when I got off track. Next, I would like to thank Betsy Brooks and the Braddock Bay Bird Observatory for their tremendous support with field work. Stacey Kieffer, Conan Guard and Leanna Bond also assisted us with some field work and I thank them. Additionally, I would like to thank Dr. Rey Sia and Dr. Paul Roberts for assistance with molecular techniques. I would also like to thank Dr. Stephen Previs and Dr. Bernard Landeau of Case Western Reserve University for granting me access to their lab and GC-MS. Furthermore, I would like to acknowledge the math/science division at Genesee Community College for loaning me the PCR machine that was critical to finishing this study. This study was supported in part by the SUNY College at Brockport Department of Environmental Science and Biology graduate student fund. Permission to take blood samples was granted by U. S. Fish and Wildlife Service Permit # MB076275-0 and New York State Fish and Wildlife License # LCP03-432.

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GENERAL INTRODUCTION

The White-throated Sparrow (*Zonotrichia albicollis*) is a North American short-distance migratory passerine (Falls and Kopachena 1994). This species has a winter range primarily south of Lake Erie to the Gulf of Mexico, while the breeding range extends north from Lake Erie and Lake Ontario, throughout much of Canada, and also includes northern New England and the Adirondack Mountains of New York (Falls and Kopachena 1994).

A unique feature of White-throated Sparrows is the existence of two distinctive plumage morphs in both sexes; white-striped (WS) and tan-striped (TS). Plumage morphs differ in the color of their crown stripes; the median and lateral crown stripes of WS birds are white whereas those of TS birds are tan or brown. Plumage morphs not only differ in their physical appearance, but also in their behavior. WS birds tend to be more aggressive, territorial, sing more, seek more extra-pair copulations and contribute less parental care toward offspring than their same sex, TS counterparts (Lowther 1961, Lowther and Falls 1968, Ficken et al. 1978, Knapton and Falls 1983, Watt et al. 1984, Kopachena and Falls 1993, Tuttle 1993, 2003).

These differences in behavior and appearance are genetically determined. Birds that possess a single copy of an inverted second chromosome are WS, while those without it are TS (Thorneycroft 1966, 1975). This plumage polymorphism is maintained by negative assortative mating. Over 95% of pairs observed on the breeding grounds consist of one WS and one TS individual (Falls and Kopachena 1994). However, WS male x TS female pairs are seen about twice as often as TS male x WS female pairs (Lowther 1961, Lowther and Falls 1968, Thorneycroft 1975, Knapton and Falls 1982,

Tuttle 1993). Pairs of either type produce half WS and half TS offspring of both sexes (Thorneycroft 1975). More observations of WS male x TS female pairs on the breeding grounds suggest that higher mortality of TS males and WS females occurs during the non-breeding season.

A major objective of my study was to compare fat reserves among plumage and sex classes of White-throated Sparrows, as differences in fat reserves may contribute to differential mortality between plumage and sex classes. Fat, and the ability to store fat, is a potentially life or death matter for many birds. Lipids are the major energy reserve for most birds (Griminger 1986). These reserves are drawn upon during times of high energy utilization, such as migration or other long distance flight (Berger and Hart 1974). Fat reserves may also be mobilized to fuel high metabolic costs of birds overwintering in cold environments (Dawson and Carey 1976). Resident diurnal birds replenish their fat stores during daylight when they are able to forage, and draw upon these stores at night when they are unable to feed (Blem 1976, 1990). Fat stores may also provide insurance against lack of foraging opportunity, whether due to weather (Carey et al. 1978, Blem and Shelor 1986, Blem 1990), or presence of predators (Lilliendahl 1998, Pravosudov and Grubb 1998). However, accumulation of excessive fat stores may result in increased wing-loading, which may reduce an individual's ability to escape predation (Lima 1986, McNamara and Houston 1990).

Various methods have been used to determine fat loads in birds. These methods can be divided into two categories, qualitative and quantitative methods. Each of these methods has advantages and drawbacks.

Qualitative methods of estimating fat stores involve making subjective measurements or using measures that are not directly related to fat stores. Probably the most commonly used qualitative method involves visually estimating subcutaneous fat stores and assigning a fat class (Helms and Drury 1960, Fransson and Jakobsson 1998, Davis 2001, Gannes 2001). Another qualitative method includes body-condition indices, which use morphological measurements to estimate fat loads, such as fat mass / wing chord length (Owen and Cook 1977, Iverson and Vohs 1982, Johnson et al. 1985, Ringleman and Szymczak 1985, Winker et al. 1992, Winker 1995). Qualitative methods of measuring fat stores have the advantage of being relatively quick, easy, and do not necessitate sacrificing the animals. However, they are not as precise as quantitative measures. Many body-condition indices are based upon morphological measures that are not related to actual fat mass carried by animals (Hayes and Shonkwiler 2001). Furthermore, these body-condition indices have rarely been validated by comparison with birds of known fat content (Conway et al. 1994). The visible fat class method is subjective, and relies on an observer's judgment, although some investigators have attempted to quantify the use of scoring fat class based upon lipid extraction from birds previously assigned a fat class (Rogers 1987, 1991, Krementz and Pendleton 1990, Kaiser 1993, Lundgren et al. 1995). Fat classes account for about 50% of variation in an individual's actual fat mass (Rogers 1987, 1991, Krementz and Pendleton 1990; but see Whitman 2002). Furthermore, Krementz and Pendleton (1990) found high variation in assinging fat class between different observers. Use of a visible fat class is also limited in that only visible fat is estimated, while any non-visible lipid stores are excluded.

Quantitative methods of measuring fat stores involve making direct or indirect, objective measurements. Examples of quantitative methods used to measure fat stores in birds include the deuterium dilution technique (Karasov and Pinshow 1998, Speakman et al. 2001, Whitman 2002) and direct chemical body composition analysis (Odum and Perkinson 1951, Odum 1960, King et al. 1965, Odum et al. 1965, Holmes 1976). Direct chemical body composition analysis is the standard quantitative means of measuring fat stores in birds, and a precise measurement of a bird's fat stores can be made by chemically extracting lipids from the bird. However, body composition analysis is time consuming and requires that birds be killed. This eliminates the possibility of following measures of an individual's body composition or fitness (such as survivorship or reproductive success) over time. Studies utilizing chemical body composition analysis and requiring a large sample size may also raise ethical concerns about killing large numbers of birds.

I used the deuterium dilution technique to estimate fat stores in migrant White-throated Sparrows. The deuterium dilution technique has advantages over most of the previously mentioned methods. It is a quantitative means to estimate fat mass, yet is quicker than chemical body composition analysis. Furthermore, once standardized, the deuterium dilution technique does not require that subjects be killed to determine body composition. Whitman (2002) validated this technique against chemical body composition analysis in White-throated Sparrows, and showed that accurate predictions of lipid stores are possible using the deuterium dilution technique. While not as quick as the visible fat class method, the deuterium dilution technique offers a precise means of estimating fat mass that is not subject to observer variability.

The deuterium (^2H) dilution technique of estimating lipid stores in an animal is based upon chemical body composition analysis. Chemical body composition analysis assumes that an animal's body is divided into three basic compartments: water, lipids, and fat-free dry matter (Fig. 1.1). Two of these compartments, body water and body lipids, do not mix, and occupy two separate and distinct compartments. In contrast, body water and fat-free dry matter are naturally found together, and represent a combined compartment referred to as fat-free wet weight.

Chemical body composition analysis works by chemically separating the three basic body components and measuring them. An animal is typically killed and then desiccated to remove water and calculate the total body water (TBW) as the difference between total body mass (TBM) and dry mass (DM) (Fig. 1.2). Lipids are extracted from the dry mass to separate the fat mass (FM) and fat-free dry mass (FFDM) body components (Fig. 1.2).

Although Pace and Rathbun (1945) suggested that total body water as a proportion of fat-free wet weight (FFWW) is constant in mammals, the TBW proportion of FFWW is not constant among different species of mammals. In their review, Sheng and Huggins (1979) reported values for the TBW proportion of FFWW from 0.63 in the beagle to 0.80 in the mouse. Part of this variation in TBW proportion of FFWW may be due to the use of different methods of chemical body composition analysis. Kerr et al. (1982) reported that researchers use many different drying temperatures and procedures to desiccate animals, while Dobush et al. (1985) found differences in the various methods and solvents used by researchers to extract lipids from animals. Although there does not appear to be a constant TBW proportion of FFWW among all species, there does appear

to be a species-specific constant TBW proportion of FFWW (Odum et al. 1964, Child and Marshall 1970, Sheng and Huggins 1979, Arnould et al. 1996).

Given a species-specific constant TBW proportion of FFWW, fat-free dry mass (FFDM) can be calculated if TBW is known. Knowledge of TBW and FFDM allows calculation of fat mass (FM), the third major body component (Fig. 1.1). The deuterium dilution technique enables TBW to be calculated, and thus body composition, including fat mass, can then be determined. A lipid index can then be calculated from fat mass and fat-free dry mass. The lipid index (g fat/g fat-free dry mass) attempts to account for the amount of fat carried by a bird relative to its body size (Odum et al. 1964, Odum et al. 1965), and represents more a measure of lipid reserves than total fat stores.

The main goal of this study was to compare fat reserves among plumage and sex classes of White-throated Sparrows. To accomplish this objective, I needed to measure fat stores, determine sex, and identify plumage morph of White-throated Sparrows.

To measure fat stores, I used the deuterium dilution technique to calculate lipid index. I then compared lipid index to fat classes (Helms and Drury 1960) assigned to migrant White-throated Sparrows of both sexes and plumage morphs (WS or TS). Additionally, I compared lipid index of identical fat classes across seasons, since this has not been investigated before.

To identify plumage and sex classes of White-throated Sparrows, I needed to identify sex of captured birds. Differences in wing chord length between males and females have been used to sex birds (United States Fish and Wildlife Service and Canadian Wildlife Service 1980, Wood and Beimborn 1981, Pyle 1997). However, there is a large overlap of wing chord lengths between the sexes (Atkinson and Ralph 1980,

Schlinger and Adler 1990, Piper and Wiley 1991, Falls and Kopachena 1994). This overlap prevents sex identification for a large number of birds. Instead of using wing chord to sex migrant White-throated Sparrows, I used molecular sexing techniques (Griffiths et al. 1998). I also evaluated the usefulness of using wing chord to identify the sex of White-throated Sparrows.

During spring migration, White-throated Sparrows are in alternate plumage, and plumage morph is easily identified based on the color of median and lateral crown stripes. However, during fall migration the birds are in basic plumage and the crown stripes are duller, making plumage identification difficult. However, Piper and Wiley (1989) developed a method to identify plumage morph of White-throated Sparrows in basic plumage. I used Piper and Wiley's (1989) technique to identify plumage morph of birds captured during fall migration.

With plumage morph and sex identified, I calculated the plumage morph frequencies for each sex during fall and spring migrations. If differential mortality occurs before or during either fall or spring migrations, I should have been able to detect more WS than TS males, and more TS than WS females, during the respective migration periods.

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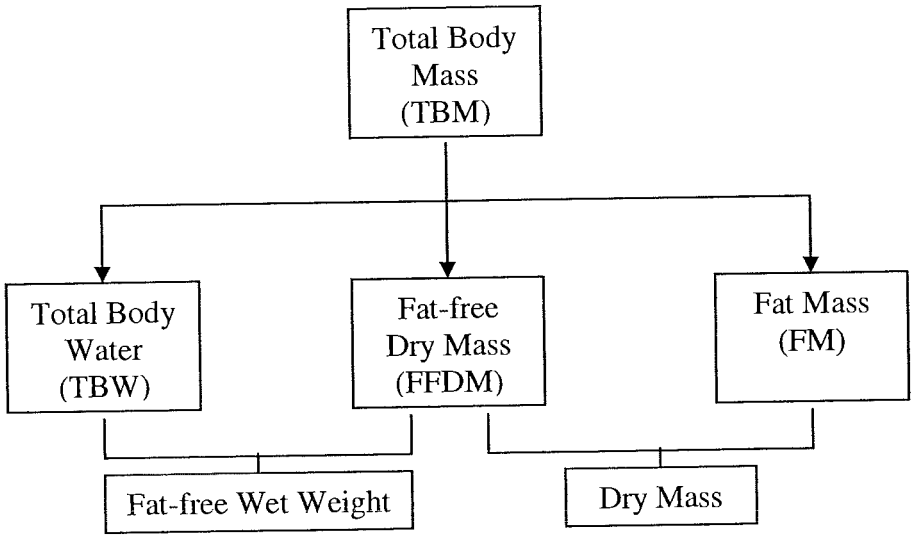

FIGURE 1.1. Flow chart of Total Body Mass components.

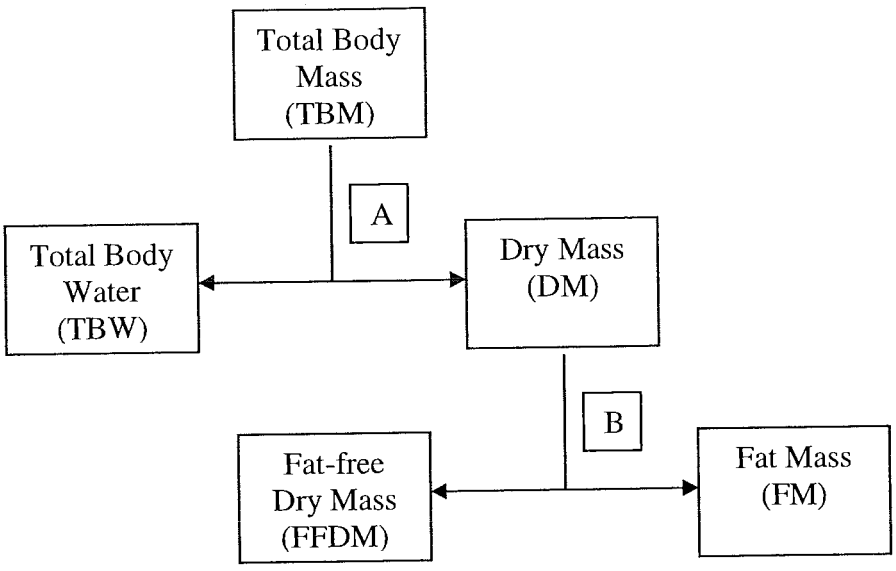

FIGURE 1.2. Flow chart of body composition analysis, where A represents desiccation and B represents extraction of lipids.

CHAPTER TWO

A between-season comparison of lipid stores carried by migrant White-throated Sparrows, as determined by deuterium dilution and visible fat class.

INTRODUCTION

For ornithologists studying wintering ecology, breeding ecology and/or migration, it is important to know the amount of fat stored by a bird (Blem 1990). The level of fat reserves present in a bird may have implications for its migration, survival and/or reproductive success (King and Farner 1966, Cherry 1982, Nolan and Ketterson 1982, Sandberg and Moore 1996). Ornithologists have frequently used visible fat classes to estimate fat stores of birds (McCabe 1943, Helms and Drury 1960).

In addition to fat classes, morphometric indices have been proposed as a means of indirectly measuring body condition (Brown 1996, Hayes and Shonkwiler 2001). However, few studies have examined the relationship between fat classes and actual fat reserves (Krementz and Pendleton 1990, Rogers 1991, Kaiser 1993, Conway et al. 1994, Lundgren et al. 1995), or between measured fat stores and morphological indices of body condition (Conway et al. 1994). Among these studies, only Rogers (1991) and Kaiser (1993) used lipid index as a measure of fat reserves. The lipid index (g fat/g fat-free dry mass) attempts to account for the amount of fat carried by a bird relative to its body size (Odum et al. 1964, Odum et al. 1965). Lipid index is a quantitative measure of stored fat, whereas fat classes and morphological indices are qualitative measures.

Qualitative and quantitative estimates of fat stores may vary with sex, age, and reproductive condition (King et al. 1965, Yarbrough 1970, Morton et al. 1973, Heise and Moore 2003), as well as temporally (Odum and Perkinson 1951, Helms and Drury 1960, Helms et al. 1967, Helms and Smythe 1969). However, I am aware of only one study comparing the relationship between fat classes and actual stored fat in fall and spring migrating birds (Lundgren et al. 1995). Using direct chemical fat extraction, Lundgren et

al. (1995) found that spring birds deposited twice the amount of fat per fat class level than did fall birds. This finding has implications for comparing fat classes between fall and spring populations.

In this study, I compared how fat stores related to visual fat classes of White-throated Sparrows (*Zonotrichia albicolis*) during fall and spring migrations. To measure fat stores, I calculated lipid index by using the deuterium dilution technique. The deuterium dilution technique accurately and precisely estimates fat mass in White-throated Sparrows (Whitman 2002) and other passerines (Karasov and Pinshow 1998). I also compared a morphometric index of body condition with lipid index across seasons.

METHODS

I conducted this study in cooperation with the Braddock Bay Bird Observatory in Greece, NY (43°19′24″N, 77°43′03″W). The observatory is located on the south shore of Lake Ontario; the habitat includes a mixture of grassland, shrubland, wetland, deciduous forest, and residential areas. I processed 144 White-throated Sparrows captured during the fall (27 September – 11 October) 2003 and spring (23 April – 12 May) 2004. Using a 27 gauge needle, I punctured the brachial vein and collected a blood sample in a micro-capillary tube coated with heparin. After the first, baseline blood sample, I gave each bird an intraperitoneal injection of 200-300 µl 99.9% $^{2}H_2O$ (Isotec, Miamisburg, OH). After the injection, I assigned each bird a fat class (0-5) based upon the relative amount of fat visible through the skin on the furculum and abdomen (Helms and Drury 1960). I held all birds in separate cloth bags for 1 hr before taking the second, enriched blood sample, in the opposite brachial vein (Whitman 2002). Before releasing each bird, I

measured its mass on an electronic balance (± 0.1 g). I transferred all blood samples to 1.5 ml Eppendorf tubes and stored them on ice while in the field. Upon returning to the lab, I centrifuged the samples, transferred the plasma to separate tubes and stored them at –20°C.

I determined the deuterium enrichment of each sample by analyzing it on a gas-chromatography mass-spectrometer (GC-MS). Since the plasma water cannot be run directly on a GC-MS, I followed a modified version of the acetone exchange technique developed by Yang et al. (1998). For details of this modified acetone exchange technique, please refer to McCabe et al. (2006).

To account for any differences in natural ^{2}H abundance between birds migrating from different latitudes, I planned to subtract the initial baseline enrichment sample from the second ^{2}H enriched sample (Chamberlain et al. 1997, Hobson and Wassenaar 1997). However, baseline samples from all birds caught in the spring or fall were the same and slightly negative. Therefore, baseline values were not subtracted from the ^{2}H enriched samples. Apparently, the instruments I used lacked the sensitivity to detect any differences in natural ^{2}H enrichment of plasma water due to latitude. Any birds calculated to have negative fat mass ($n = 4$) were excluded from analysis. I calculated total body fat using the following equation developed for White-throated Sparrows in a validation study by Whitman (2002):

Eq. 1. fat mass (g) = total body mass (g) – ^{2}H dilution space (ml) – [^{2}H dilution space (ml) / 2.885].

To calculate ^{2}H dilution space, I employed the following equations (McCabe et al. 2006):

Eq. 2. ^{2}H dilution space (ml) = $^{2}H_2O$ injected (ml) / absolute ^{2}H enrichment of plasma

Eq. 3. absolute ^{2}H enrichment of plasma = [m/z 59 / (m/z 58 + m/z 59) – b] / m

Where m/z 59 is the mass spectrometry (MS) signal for ^{2}H labeled acetone and m/z 58 is the MS signal for unlabeled acetone, b = y-intercept of the standard curve, and m = the slope of the standard curve. Terms b and m are used to correct the plasma water ^{2}H enrichment against the standard curve of natural ^{2}H enrichment of water.

After calculating total body fat, I determined a lipid index for each bird to account for the amount of fat carried relative to body size (Odum et al. 1964, Odum et al. 1965, Owen and Cook 1977).

Eq. 4. lipid index = fat mass / fat-free dry mass

In order to calculate fat-free dry mass I used the following simple subtraction:

Eq. 5. fat-free dry mass = total body mass – (water mass + fat mass)

I used the following equation based upon Child and Marshall (1970) to calculate water mass:

Eq. 6. water mass = fat-free mass * 0.6785

Where 0.6785 is the combined mean water ratio for White-throated Sparrows in sub-groups A and B as determined by Child and Marshall (1970).

Additionally, I also calculated a condition index for each bird (Winker 1995):

Eq. 7. condition index (CI) = 100 * mass / wing chord

STATISTICAL ANALYSIS

Due to the small sample sizes of fat class categories 4 ($n = 4$) and 5 ($n = 2$), I pooled these data into a single category. Since condition index was normally distributed for each fat class category, I examined the relationship between fat class and condition index with

an ANOVA test. To find differences between specific fat class levels, I used Tukey multiple comparison tests. Because lipid index values for some fat class categories were not normally distributed, I transformed these data (Blem 1984). However, after transforming these data lipid index values were still not normally distributed for some fat class categories. Thus, I assessed the relationship between fat class and lipid index using a Kruskal-Wallis test. For the association between lipid index and condition index, I used linear regression analysis. Additionally, I tested for seasonal differences in lipid index and condition index using t-tests. Both lipid index and condition index were normally distributed for each season. Due to the ordinal nature of the data, I tested for differences in fat classes with a Mann-Whitney U test. Following Krementz and Pendleton (1990) and Rogers (1991) I used linear regression to test the relationship between lipid index and fat class separately for fall and spring birds. Then I compared slopes between seasons with a t-test (Zar 1999). Finally, I used a general linear model to test the effect of season, fat class and the interaction of season and fat class on lipid index. I used the Minitab statistical program version 14.12.0 (Minitab 2004) to perform all statistical tests according to Zar (1999). All tests were two-tailed and considered significant at the 0.05 level of significance; values reported are means or medians ± SE.

RESULTS

White-throated Sparrows with different fat classes had significantly different lipid index values ($H_4 = 60.0$, $P < 0.001$, Fig. 2.1). Individuals with different fat classes also had significantly different condition index values (ANOVA, $F_{4, 139} = 31.4$, $P < 0.001$, Fig. 2.2). Condition index values differed significantly between all fat class categories except

0 and 1. Linear regression analysis revealed a significant association between condition index and lipid index values ($r = 0.708$, $P < 0.001$).

In testing for seasonal differences in migrating birds, I found significantly higher fat class ($U_{61, 83} = 3581$, $P < 0.001$), lipid index ($t_{140} = 14.62$, $P < 0.001$), and condition index ($t_{140} = 6.34$, $P < 0.001$) in spring migrant White-throated Sparrows than in fall migrants (Table 2.1). Within fat classes, lipid index (Table 2.2), and to some extent condition index (Table 2.3), was significantly higher in spring versus fall birds.

The linear regression relationship between fat class and lipid index was positive and significant for both fall ($r = 0.643$, $P < 0.001$, Fig. 2.3) and spring ($r = 0.762$, $P < 0.001$, Fig. 2.3). But the spring slope was significantly steeper than the fall slope ($t_{140} = 7.53$, $P < 0.001$, Fig. 2.3). Although both fat class and season significantly affected lipid index, interpretation of the relationship between each predictor variable and lipid index is complicated by the significant interaction term (Table 2.4). The linear regression relationship between lipid index and condition index was also positive and significant for both fall ($r = 0.449$, $P < 0.001$, Fig. 2.4) and spring ($r = 0.688$, $P < 0.001$, Fig. 2.4). But the spring slope was significantly steeper than the fall slope ($t_{140} = 4.18$, $P < 0.001$, Fig. 2.4).

DISCUSSION

Median lipid index increased between successive fat class levels. However, there were large overlaps in lipid index values between neighboring fat class intervals (Fig. 2.1). The increase in median lipid index varied from 0.18 to 0.48 between adjacent fat class levels. Rogers (1991) recorded similar, though smaller, differences in lipid index

between fat classes for Dark-eyed Juncos (*Junco hyemalis*). Kaiser (1993) also reported that the relationship between fat class and lipid index was linear for lean and medium fat birds, while it was exponential for fatter birds. These results support Hailman's (1965, 1969) assertion that fat class data are of an ordinal, and not an interval, scale.

Mean condition index also increased between most successive fat class levels. However, condition index values for fat class levels 0 and 1 were not significantly different. As with lipid index, there was large overlap in condition index values between adjacent fat class levels.

The fact that lipid index and condition index were significantly related to each other revealed that a simple equation using easily obtained morphometric measurements could shed light upon the fat status of a migratory passerine. However, the equation for condition index only explained half of the variation in lipid index values among individuals.

While individuals with fat class of 0 had no visible subcutaneous fat, they did register a median lipid index of 0.39, indicating the presence of unseen fat reserves. Thus, a 24 g bird with 7 g of fat-free dry mass and a fat class of 0 should carry about 2.6 grams of fat. Rogers (1991) also found that wintering Dark-eyed Juncos with a 0 fat class had a lipid index well above those individuals known to have depleted their fat reserves.

I found that spring migrant White-throated Sparrows had higher lipid index values, and thus carried higher fat loads, than fall migrants. Kuenzel and Helms (1974) found similar results with respect to fat class only for spring and fall migrant White-throated Sparrows. Furthermore, I found that for a given fat class, spring birds had a

significantly higher lipid index than did fall birds. Lundgren et al. (1995) also found that for the same fat class, spring migrating Willow Warblers (*Phylloscopus trochilus*) carried more fat than fall migrating birds. I am not aware of other studies that have reported this trend, as most attempts to quantify fat classes have only employed birds from a single season. Kaiser (1993) captured birds only during fall, while Krementz and Pendleton (1990) and Rogers (1991) only captured birds during winter. Lundgren et al. (1995) suggested that this difference might be related to how stored fat is distributed through a bird's body.

Although subcutaneous deposits are the primary location for lipid stores in White-throated Sparrows and other birds (Odum and Perkinson 1951, Blem 1976, 1990), extra fat for migration is stored throughout the body, except the heart (Odum and Perkinson 1951). However, these fat deposits in White-throated Sparrows show different magnitudes of storage and degrees of change prior to migration (Odum and Perkinson 1951). While subcutaneous fat deposits comprise the largest area of fat storage in White-throated Sparrows, peritoneal fat exhibits the largest relative increase (5 to 14% of total stored fat) prior to migration (Odum and Perkinson 1951). Together, subcutaneous (43%) and peritoneal (14%) fat deposits make up the majority of total stored lipids in premigratory spring White-throated Sparrows (Odum and Perkinson 1951). However, only subcutaneous, and not intraperitoneal fat stores, can be partially seen and evaluated by visual examination of the furculum and abdomen, as per Helms and Drury (1960). Thus, intraperitoneal fat, and fat stored in other body areas (liver, muscle, viscera, and un-evaluated subcutaneous deposits), are left unexamined by visual inspection. Variation in these fat storage locations may also contribute to seasonal differences seen in lipid

index values for White-throated Sparrows with identical fat classes. Odum and Perkinson (1951) examined some of these other body areas in White-throated Sparrows prior to spring migration and found the following percentages of total stored fat in each: head and neck 7%, thorax 15%, posterior 9%, viscera 9%, heart 1%, and liver 2%. Liver and muscle contain very little fat in both seabirds and White-throated Sparrows, and therefore are unlikely to contribute to seasonal differences in lipid index for birds with identical fat classes (Odum and Perkinson 1951, Osborn and Harris 1984). Liver fat as a percent of total stored fat actually decreased (from 5 to 2%) between pre-alternate molt and the spring premigratory period (Odum and Perkinson 1951). White-throated Sparrows do differ in where lipid is deposited during the winter and spring migratory periods (Odum and Perkinson 1951, Pond 1978); while mostly subcutaneous fat is stored for spring migration and during winter, much more fat is stored within the peritoneum for spring migration than during winter (Odum and Perkinson 1951).

As another explanation for why spring Willow Warblers had higher lipid index values for a given fat class than fall migrants, Lundgren et al. (1995) suggested that birds may be in a different stage of migration when captured in different seasons, at the same location. White-throated Sparrows arriving at Braddock Bay in the spring could be in a different stage of migration than those that arrive in the fall. Braddock Bay may not be equidistant between wintering and breeding areas, resulting in birds that are further into migration during one season compared to the other. Caldwell et al. (1964) reported that the amount of fat reserves carried by White-throated Sparrows varies with stage of migration. Thus, if White-throated Sparrows arrive at Braddock Bay in a different stage of migration in spring than fall, then they may carry different amounts of fat reserves

when they are at Braddock Bay during the different seasons. Migrant White-throated Sparrows also exhibit seasonal differences, as they are heavier and have higher fat classes in spring (Kuenzel and Helms 1974, Falls and Kopachena 1994) and migrate more rapidly with shorter stopovers in spring than fall (Borror 1948). As Braddock Bay is located on the southern shore of Lake Ontario, the proximity of this large body of water could influence fat deposition. Fall migrants would have flown at least 70 km across the lake, and therefore be expected to carry less fat due to fuel consumption. If fat were not burned evenly from fat deposits, this could lead to mis-estimation of fat stores (Lundgren et al. 1995). This is plausible because birds may store subcutaneous fat before intraperitoneal fat, but utilize intraperitoneal fat before subcutaneous fat (Blem 1990).

The significant interaction between fat class and season on lipid index is important. Not only do identical fat classes from different seasons represent different degrees of fat storage, but the rate of change between fat class levels also differs between seasons. The degree of increase between fat class levels in spring White-throated Sparrows was twice that of fall birds. Lundgren et al. (1995) observed that Willow Warblers also deposited twice as much fat per fat class level than did fall birds. Furthermore, lipid index for a White-throated Sparrow with a fat class of 0 in spring was twice that of a fall bird. Because fat classes do not consistently estimate fat storage between seasons, I advise against comparing fat classes between different seasons.

There was much variation in lipid index values both within and among fat classes (Fig. 2.1). Some of this variation may have been due to error from the deuterium dilution technique and associated equations used to calculate lipid index and some variation may have been due to problems with assigning fat class. Validations of deuterium dilution

analyses have shown that it can accurately and precisely estimate fat mass (Karasov and Pinshow 1998, Whitman 2002). Whitman's (2002) equation (Eq. 1) estimated fat mass with a standard error of 1.08 g for her calibration group. Whitman (2002) then tested the predictive equation against a validation group that was not included in developing this equation and found that the predictive equation estimated fat mass with a relative error of 28.42 ± 12.46% (SE). To calculate fat mass using Whitman's (2002) equation, deuterium dilution space must be known. I used the acetone-exchange method of Yang et al. (1998) to measure deuterium dilution space of White-throated Sparrows using GC-MS instead of following Whitman (2002), who followed Karasov et al. (1988). McCabe et al. (2006) investigated the reproducibility of the acetone-exchange method for measuring deuterium labeled samples and found the coefficients of variation of the assay to usually be less than 0.5%.

As I used lipid index (Eq. 4) and not fat mass for my comparisons, I relied on the mean water ratios calculated by Child and Marshall (1970) to calculate water mass. I then could subtract both water mass and fat mass from total body mass to calculate fat-free mass (Eq. 5). Child and Marshal (1970) calculated mean water ratios for two groups of White-throated Sparrows collected during fall migration. The two groups had mean water ratios (water as a percentage of fat-free mass) of 67.87 ± 0.54% (99% CI) and 67.83 ± 0.51% (99% CI), respectively. For my calculations, I combined the two water ratios into a single term and converted it from a percentage to a proportion.

Assigning a visible fat class can be a reasonable, qualitative means of estimating fat reserves of White-throated Sparrows and other passerines (Krementz and Pendleton 1990, Conway et al. 1994). However, fat classes do not measure the total fat reserves of

a bird, since the scoring system is based only on visible subcutaneous fat. Furthermore, Krementz and Pendleton (1990) observed that considerable variation in assigning fat classes exists between different observers. Therefore, fat classes should be used only when a rough estimate of fat reserves is needed and efforts have been made to minimize both inter-observer and intra-observer error. Wiersma and Piersma (1995) also suggested that fat classes are best used for comparing fat content among groups instead of individuals. When more accurate and specific data than fat classes of individuals are required, quantitative methods such as deuterium dilution analysis or direct chemical analysis should be employed.

Deuterium dilution analysis is often preferable to direct chemical analysis. Once validated, deuterium dilution analysis does not require killing birds to measure their fat stores, as is the case with direct chemical analysis. In addition to being a more humane treatment of subjects, deuterium dilution analysis allows for repeated measurements of individuals over time. Additionally, the laboratory effort required for deuterium dilution analysis is less time consuming and less messy than direct chemical analysis. One proposed disadvantage of the deuterium dilution technique to estimate fat mass in songbirds is the required laboratory effort involved to prepare samples for infrared spectrophotometry with micro-distillation (Karasov et al. 1988, Karasov and Pinshow 1998, Whitman 2002). By using the acetone exchange method (Yang et al. 1998, McCabe et al. 2006) to measure deuterium enrichment of my samples, I reduced and simplified the laboratory effort necessary to perform the deuterium dilution technique. With access to a GC-MS, this makes the deuterium dilution technique a very attractive means of quantifying fat stores in passerines.

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TABLE 2.1. Median fat class, mean mass, mean lipid index [fat mass (g) / fat-free dry mass (g)] and mean condition index [100 * total mass (g) / wing chord length (mm)] for migrating White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004. All median and mean values are ± SE.

	Fall	Spring
Fat Class	1.0 ± 0.2	2.0 ± 0.1
Mass	24.2 ± 0.2	25.7 ± 0.2
Lipid Index	0.46 ± 0.03	1.06 ± 0.03
Condition Index	34.27 ± 0.24	36.71 ± 0.30

TABLE 2.2. Median lipid index [fat mass (g) / fat-free dry mass (g)] values ± SE for fall and spring migrating White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004. The range of lipid index values for each season's fat class is shown in parentheses underneath the median. I used Mann-Whitney U-tests to compare fall and spring lipid index values within each fat class.

	Fall		Spring		
Fat Class	Median Lipid Index (range)	*n*	Median Lipid Index (range)	*n*	*P*
0	0.28 ± 0.03 (0.15 – 0.49)	17	0.69 ± 0.05 (0.45 – 0.74)	6	0.001
1	0.45 ± 0.05 (0.04 – 0.72)	18	0.83 ± 0.04 (0.54 – 1.12)	17	< 0.001
2	0.45 ± 0.04 (0.27 – 0.75)	11	1.02 ± 0.04 (0.58 – 1.42)	27	< 0.001
3	0.64 ± 0.04 (0.42 – 0.92)	14	1.28 ± 0.04 (0.65 – 1.69)	28	< 0.001
4 & 5	0.71 (N/A)	1	1.60 ± 0.13 (1.04 – 1.79)	5	N/A

TABLE 2.3. Median condition index [100 * total mass (g) / wing chord length (mm)] values ± SE for fall and spring migrating White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004. The range of condition index values for each season's fat class is shown in parentheses underneath the median. We used Mann-Whitney U-tests to compare fall and spring condition index values within each fat class.

	Fall		Spring		
Fat Class	Median Condition Index (range)	*n*	Median Condition Index (range)	*n*	*P*
0	33.42 ± 0.34 (30.77 – 35.43)	17	35.52 ± 1.04 (0.45 – 0.74)	6	N.S.
1	33.68 ± 0.32 (30.00 – 35.63)	18	34.31 ± 0.47 (30.61 – 36.76)	17	N.S.
2	34.67 ± 0.69 (31.29 – 38.36)	11	36.35 ± 0.28 (33.80 – 39.59)	27	0.046
3	35.60 ± 0.35 (32.57 – 37.73)	14	38.02 ± 0.41 (34.86 – 43.64)	28	< 0.001
4 & 5	38.38 (N/A)	1	42.21 ± 1.29 (38.21 – 44.43)	5	N/A

TABLE 2.4. General Linear Model table showing how fat class, season and the interaction between fat class and season affect Lipid Index. SD = 0.1812, $R^2 = 0.8050$.

Source	df	Seq. SS	Adj. SS	Adj. MS	*F*	*P*
Season	1	12.8777	4.1003	4.1003	124.89	< 0.001
Fat Class	4	4.8438	3.9331	0.9833	29.95	< 0.001
Season * Fat Class	4	0.4427	0.4427	0.1107	3.37	0.012
Error	134	4.3994	4.3994	0.0328		
Total	143	22.5636				

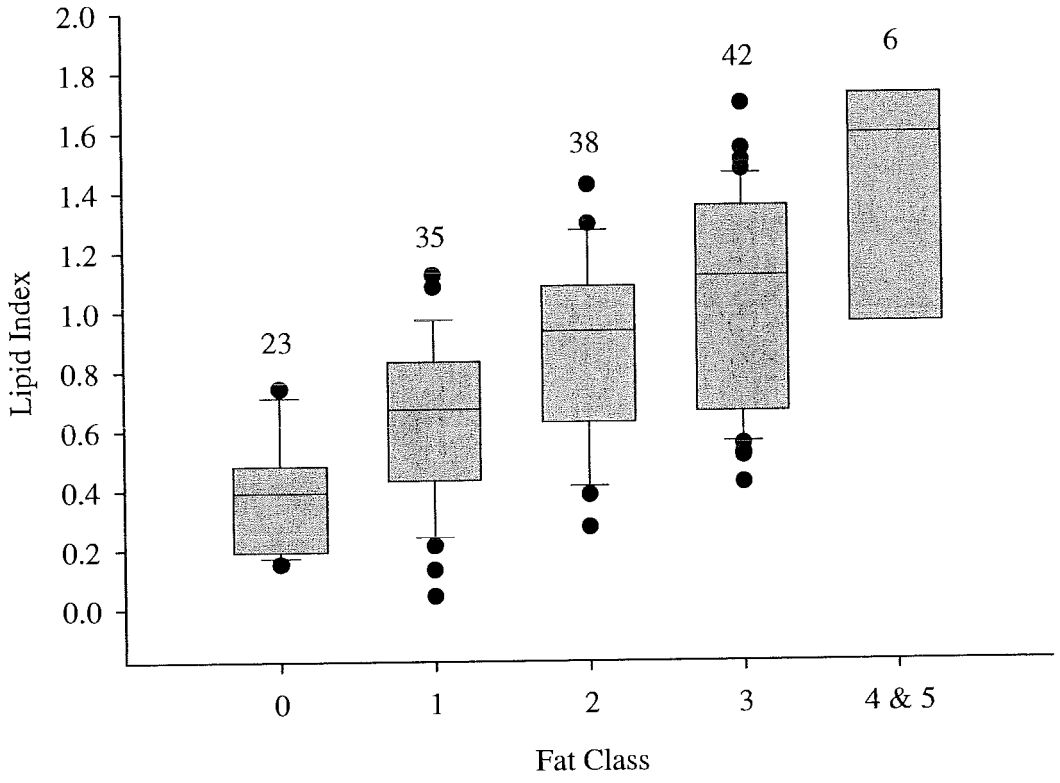

FIGURE 2.1. Lipid Index (fat mass / fat-free dry mass) for White-throated Sparrows with different fat classes, captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004 migration. The horizontal line within each gray box is the median. The lower border of the box is the 1st quartile and the upper border of the box is the 3rd quartile. The gray box therefore represents the middle 50% of values. The whiskers (error bars) extend 1.5 * interquartile range (3rd quartile – 1st quartile) above and below 3rd and 1st quartiles, respectively. Dots show values that lay beyond these whiskers. Sample size is shown above each plot.

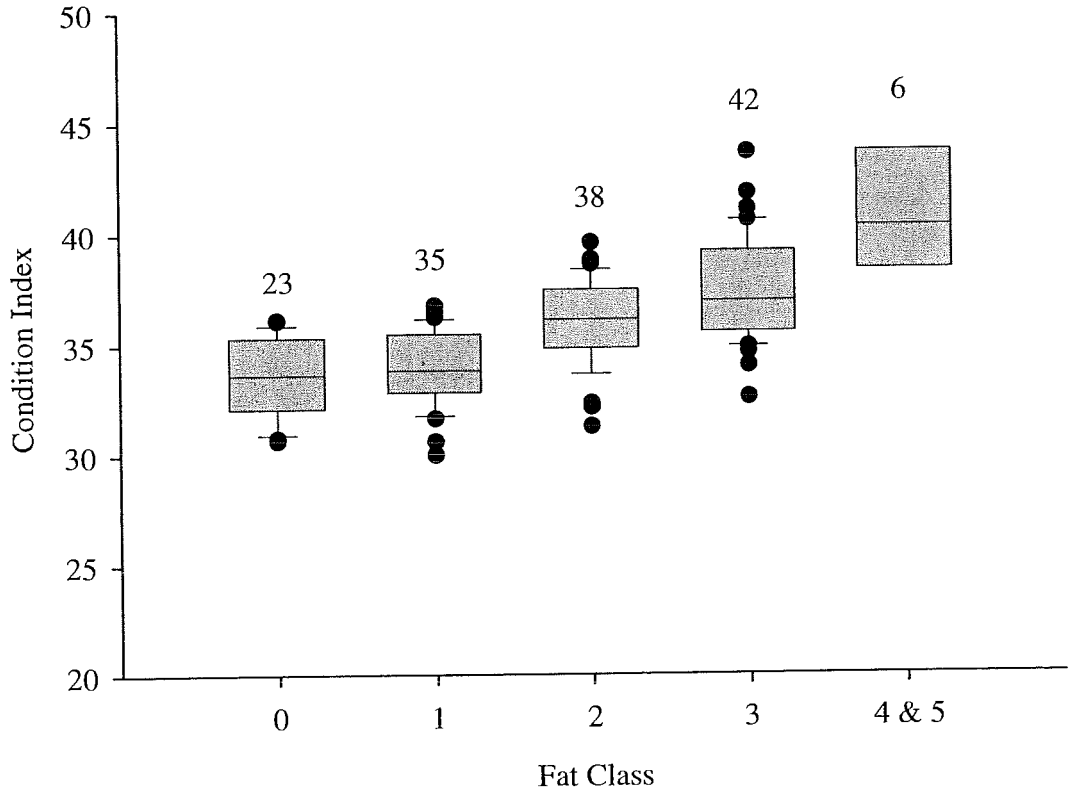

FIGURE 2.2. Box plot of Condition Index (100 * total mass / wing chord) for White-throated Sparrows with different fat classes, captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004 migration. The horizontal line within each gray box is the median. The lower border of the box is the 1st quartile and the upper border of the box is the 3rd quartile. The gray box therefore represents the middle 50% of values. The whiskers (error bars) extend 1.5 * interquartile range (3rd quartile – 1st quartile) above and below 3rd and 1st quartiles, respectively. Dots show values that lay beyond these whiskers. Sample size is shown above each plot.

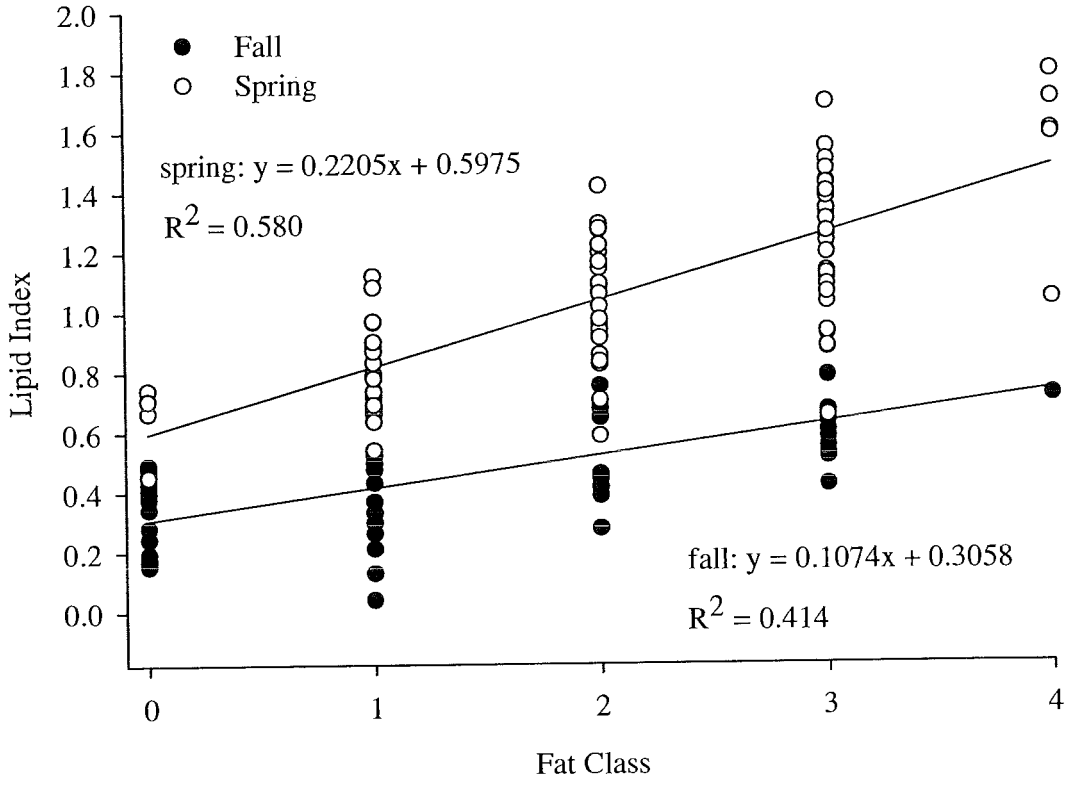

FIGURE 2.3. Relationship between Lipid Index [fat mass (g) / fat-free dry mass (g)] and fat class for White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004.

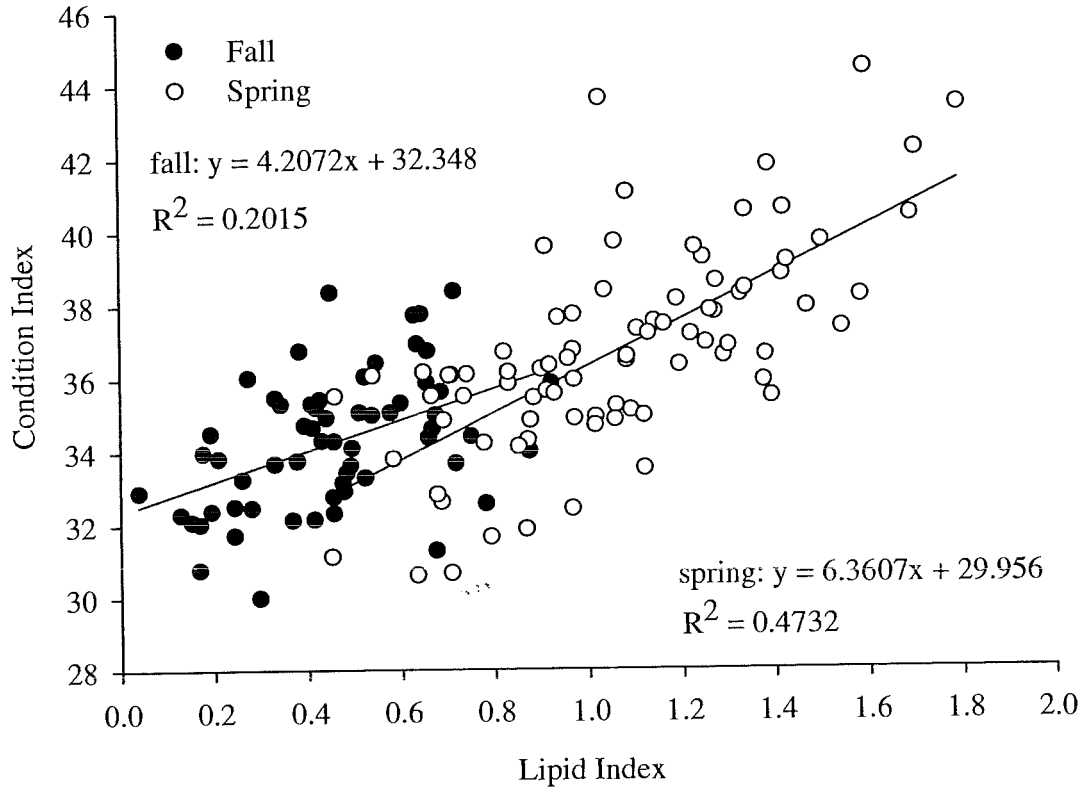

FIGURE 2.4. Association between Lipid Index [fat mass (g) / fat-free dry mass (g)] and Condition Index [100 * total mass (g) / wing chord (mm)] for White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during fall 2003 and spring 2004.

CHAPTER THREE

Is wing chord a useful criterion to sex White-throated Sparrows?

INTRODUCTION

Determining the sex of White-throated Sparrows (*Zonotrichia albicollis*) and other *Zonotrichia* outside of the breeding season is difficult. Traditionally, laparotomy (see Bailey 1953, Risser 1971 for detailed description of this technique) was the only definitive way to determine the sex of any individual of this species outside of the breeding season. Despite studies that have shown laparotomies do not affect behavior (Piper and Wiley 1991), condition (Piper and Wiley 1991) or survival (Ketterson and Nolan 1986, Piper and Wiley 1991), laparotomies are surgical procedures that carry an inherent risk for any bird. Recent work in molecular biology has given us a new (and less invasive) way to positively identify sex in birds (Ellergren 1996, Griffiths et al. 1996, 1998). However, not all researchers have the equipment, expertise or time to utilize molecular sexing techniques.

Distinguishing the sexes based upon differences in wing chord length is a quicker and simpler method than laparotomy or molecular sexing. Previous studies of White-throated Sparrows found that males tend to have larger wing chord lengths compared to females, but with substantial overlap (Atkinson and Ralph 1980, Schlinger and Adler 1990, Piper and Wiley 1991, Falls and Kopachena 1994). Due to the tendency of some males to have longer wing chord measurements than females, some sources have developed cutoff values to determine the sex of White-throated Sparrows (United States Fish and Wildlife Service and Canadian Wildlife Service 1980, Wood and Beimborn 1981, Pyle 1997). Birds with wing chord measurements larger than a given value have been classified as males, whereas birds with measurements smaller than another value have been classified as females. However, the large degree of overlap in wing chord

measurements between male and female White-throated Sparrows did not allow all, or even most, individuals to be sexed. Despite the overlap, the United States Fish and Wildlife Service [USFWS] and Canadian Wildlife Service [CWS] (1980) and Pyle (1997) advocated using wing chord length to determine sex in White-throated Sparrows. However, these authorities disagree on the wing chord values used to distinguish the sexes. The USFWS and CWS (1980) stated that White-throated Sparrows with wing chords ≤ 67 mm were females and all birds with wing chords ≥ 74 mm were males. Alternatively, Pyle (1997) proposed that birds with wing chords > 72 mm were males and those with wing chords < 69 mm were females.

I used the molecular techniques of Griffiths et al. (1998), combined with the direct PCR techniques of Bercovich et al. (1999) and Tomasulo et al. (2002), to determine the sex of White-throated Sparrows captured during fall and spring migration in western New York. I then compared the wing chord lengths of males and females in order to test the usefulness and limits of wing chord length as a criterion for sexing. Additionally, I examined the accuracy of the wing chord values proposed by the USFWS and CWS (1980) and Pyle (1997) for sexing White-throated Sparrows.

METHODS

During fall (27 September – 11 October) 2003 and spring (23 April – 12 May) 2004, I obtained blood samples and wing chord measurements from 159 White-throated Sparrows captured at Braddock Bay Bird Observatory (BBBO), Greece, NY (43°19′24″N, 77°43′03″W). BBBO personnel made all un-flattened wing chord measurements as described by Pyle (1997). I used a 27 gauge needle to pierce the

brachial vein and collect a sample of blood in a micro-capillary tube coated with heparin. After collection, I transferred the blood samples to1.5 ml Eppendorf tubes and stored them on ice until I returned to the lab where I separated the plasma and pellet portions using a micro-centrifuge and stored the two components separately at –20°C for later analysis. The plasma component was used for a separate study of lipid stores in different plumage and sex classes of White-throated Sparrows.

Following the molecular methods of Griffiths et al. (1998), I determined the sex of each bird. However, I employed direct polymerase chain reaction (PCR) techniques using a modified version of Bercovich et al. (1999) and Tomasulo et al. (2002). I substituted a 1% suspension of red blood cells from the pellet for diluted whole blood. Following the manufacturer's (Takara Inc.) recommendations, I prepared the PCR reactions using 5 µl of 1% pellet suspension in a 25 µl final reaction volume. As per Bercovich et al. (1999), I added 0.625 units of Taq polymerase after the initial denaturation cycle. Using a Bio-Rad Gene Cycler™ thermal cycler, I amplified the DNA fragments. Using gel electrophoresis, I then ran the PCR products through a 3% agarose gel for at least 1 hr to separate the CHD-W and CHD-Z bands. One band indicated a male, while two bands indicated a female (Griffiths et al. 1998).

STATISTICAL ANALYSIS

I tested for differences in means of male and female wing chord lengths using an independent two-sample t-test. Additionally, I tested for differences in mean wing chord length between fall and spring captured birds using an independent two-sample t-test. I used the Minitab statistical program version 14.12.0 (Minitab 2004) to perform all

statistical tests according to Zar (1999). All tests were two-tailed and considered significant at the 0.05 level of significance; values reported are means ± SE.

RESULTS

Mean male wing chord ($\bar{x}$ = 72.8 ± 0.2 mm, range 67.0 – 78.0, n = 74) was significantly larger (t_{148} = 16.27, $P < 0.001$) than mean female wing chord ($\bar{x}$ = 68.3 ± 0.2 mm, range 64.0 – 73.0, n = 85). There was no wing chord length difference between fall males ($\bar{x}$ = 72.7 ± 0.3, n = 38) and spring males ($\bar{x}$ = 72.9 ± 0.3, n = 36) (t_{68} = 0.32, P = 0.747) or between fall females ($\bar{x}$ = 68.4 ± 0.3, n = 36) and spring females ($\bar{x}$ = 68.2 ± 0.2, n = 49) (t_{74} = 0.66, P = 0.51). The smallest male had a wing chord of 67.0 mm, while the largest female measured 73.0 mm (Fig. 3.1). All birds with wing chord measurements ≥ 73.5 mm were males, while all individuals with wing chord measurements ≤ 66.5 mm were females. However, using these values only 42% of males (31 of 74) could safely be separated from all females and only 16% of females (14 of 85) could safely be separated from all males, based solely on non-overlapping wing chord length (Table 3.1). The proportion of overlap differed significantly between the sexes (χ^2_1 = 12.6, $P < 0.001$; Table 3.1), with females overlapping males to a greater degree than vice versa. Of the males, 97% had wing chord measurements between 69.0 and 78.0 mm, while 99% of females had wing chord measurements between 64.0 and 72.0 mm.

DISCUSSION

There was a bimodal distribution of wing chord measurements between male and female White-throated Sparrows. However, there was a high degree of overlap between male

and female wing chord measurements. The overlap observed in this study was similar to that seen in other studies (Schlinger and Adler 1990, Piper and Wiley 1991). However, I did observe males with smaller wing chord lengths (< 69 mm) and females with larger wing chord lengths (> 72 mm) than has been found elsewhere (Atkinson and Ralph 1980, Pyle 1997). Additionally, Schlinger and Adler (1990) observed several female White-throated Sparrows with wing chord lengths greater than those I measured.

In this study, male wing chord lengths ranged between 67.0 and 78.0 mm, and female wing chord lengths fell between 64.0 and 73.0 mm (Fig. 3.1). Although mean wing chord lengths differed significantly between the sexes, there was a large degree of overlap, with 58% of males and 84% of females having wing chord lengths within the observed range of the opposite sex; therefore, sex could not be readily determined by wing chord length for the majority (72%) of White-throated Sparrows that I captured. Pyle (1997) stated that male White-throated Sparrow wing chord lengths are between 69 and 78 mm, and female wing chord lengths are between 64 and 72 mm. Falls and Kopachena (1994) reported adult male wing lengths between 72 and 77.8 mm, and adult female wing lengths between 65.4 and 73.9 mm. The ranges given by Pyle (1997) to identify sex of White-throated Sparrows (> 72 mm = male, < 69 mm = female) would have correctly sexed 65% of males and 56% of females captured and measured in this study. However, these same wing chord criteria would have incorrectly sexed 3% of males and 1% of females, while 32% of male and 42% of female White-throated Sparrows captured in this study could not have been sexed by using wing chord length.

Piper and Wiley (1991) suggested different criteria than Pyle (1997) for sexing White-throated Sparrows by wing chord. The different sets of wing chord lengths

represented varying levels of compromise between comprehensive and accurate sex identification. The values (≤ 70.0 mm = female, > 70.0 mm = male) that were most comprehensive, and thus could assign a sex to all individuals, resulted in the least accuracy of sex identification for birds in this study (93% of females correctly identified, 88% of males correctly identified). However, the values (≤ 66.0 mm = female, ≥ 74.0 mm = male) that had the greatest accuracy (100% of females and males correctly assigned) resulted in the least comprehensive sexing, and could only be used to sex 34% of males and 13% of females in this study. Using intermediate criteria (≤ 67.0 mm = female, ≥ 73.0 mm = male) results in a tradeoff between comprehensive and accurate sex identification. In this study, 61% of males and 29% of females could be identified using the intermediate criteria; 1% of males and 1% of females would also have been misidentified. Additionally, 38% of males and 69% of females would have remained unidentified. Schlinger and Adler (1990) also developed a logistic regression model using wing chord length and throat line scores that could assign sex, although addition of throat line scores increased the predictive model's accuracy by only 1% over the model using only wing chord.

The USFWS and CWS (1980) have designated all White-throated Sparrows with a wing chord ≤ 67 mm as females and all White-throated Sparrows with a wing chord ≥ 74 mm as males. The use of this classification scheme would not have resulted in any misidentification of sex for birds in this study, although it could have been used to sex only 16% of males and 16% of females that I captured. Recently the Bird Banding Laboratory adopted Pyle's (1997) manual to age and sex all North American Passerines, including White-throated Sparrows (Tautin 1998). Although Pyle's (1997) wing chord

criteria for sexing White-throated Sparrows can identify sex for more birds, these criteria also misidentify more birds than the USFWS and CWS (1980) criteria. This could make if difficult to investigate fields such as differential migration (Jenkins and Cristol 2002). Therefore, I would recommend using the USFWS and CWS (1980) criteria for sexing White-throated Sparrows by wing chord when it is not acceptable for any birds to be misidentified and molecular sexing capabilities are not available. However, when all birds in a study need to be accurately sexed, the molecular sexing techniques developed by Griffiths et al. (1998) should be used instead. With the development of direct PCR techniques, including the methods described in this paper, molecular sexing techniques for birds are quicker and simpler than they were just a few years ago. Depending on experience, available equipment, and number of blood samples processed at a time, one could spend as little as 15 minutes of lab time and less than $2.00 on supplies for each bird sexed, as in this study.

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TABLE 3.1. Overlap of wing chord measurements between male and female White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during Fall 2003 and Spring 2004.

Sex	No Overlap	Overlap	*n*
Males	31 (42%)	43 (58%)	74
Females	14 (16%)	71 (84%)	85

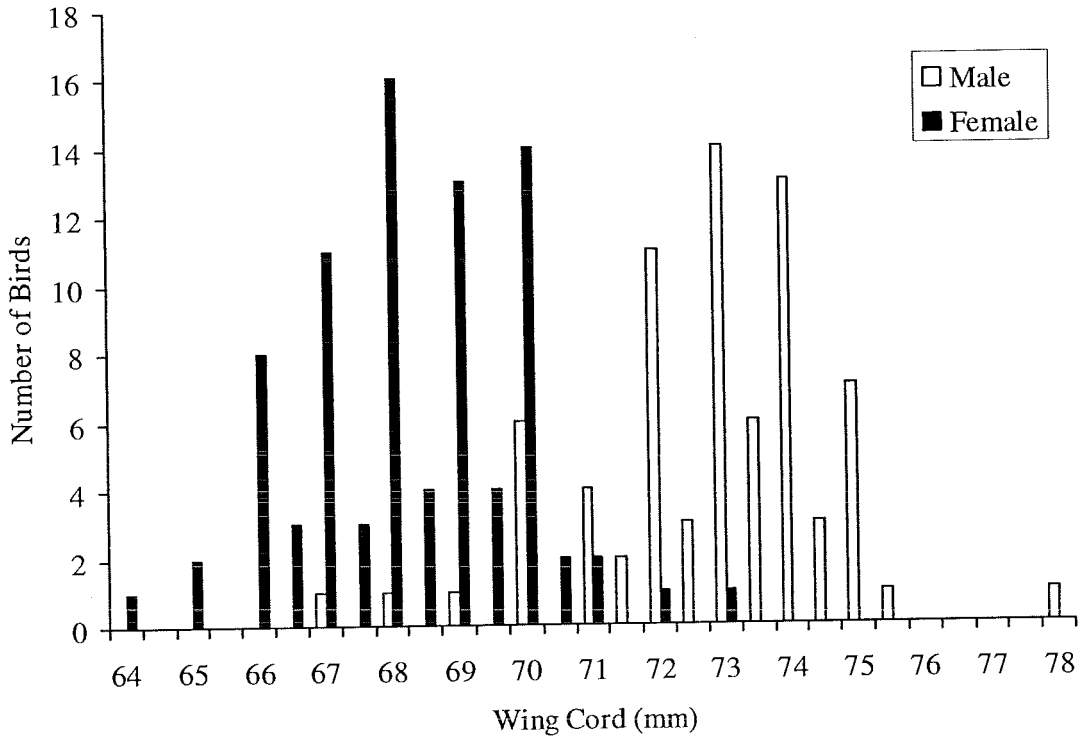

FIGURE 3.1. Distribution of wing chord measurements for male (n = 74) and female (n = 85) White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during Fall 2003 and Spring 2004.

CHAPTER FOUR

Does lipid index differ between plumage and sex classes of migrant White-throated Sparrows?

INTRODUCTION

The White-throated Sparrow (*Zonotrichia albicollis*) is a North American short distance migratory passerine. Its breeding grounds are primarily in Canada and its wintering areas are mostly in the southeastern United States (Falls and Kopachena 1994). During the breeding season White-throated Sparrows show a plumage polymorphism, displaying either white or tan colored crown stripes. Each plumage morph also exhibits different behaviors. Generally, white-striped (WS) birds are more aggressive, territorial, sing more, seek more extra-pair copulations and contribute less parental care toward offspring than their same sex, tan-striped (TS) counterparts (Lowther 1961, Lowther and Falls 1968, Ficken et al. 1978, Knapton and Falls 1983, Watt et al. 1984, Kopachena and Falls 1993, Tuttle 1993, 2003). These plumage and behavioral characteristics are genetically determined by a chromosomal inversion (Thorneycroft 1966, 1975). WS birds are heterozygous for the inverted second chromosome, while TS birds are homozygous and do not possess an inverted second chromosome (Thorneycroft 1966, 1975).

This plumage polymorphism is sustained by negative assortative mating between plumage morphs (Lowther 1961, Lowther and Falls 1968, Thorneycroft 1975, Knapton and Falls 1982, Tuttle 1993, Formica et al. 2004). WS male x TS female pairs and TS male x WS female pairs produce equal proportions of offspring for all plumage and sex classes (Thorneycroft 1975). However, observations of breeding pairs indicate that WS male x TS female pairs are more common than TS male x WS female pairs by a ratio of approximately 2:1 (Lowther 1961, Lowther and Falls 1968, Thorneycroft 1975, Knapton and Falls 1982, Tuttle 1993). Differential mortality between the plumage and sex classes

may account for these dissimilar plumage morph frequencies (Falls and Kopachena 1994).

Differential mortality may occur on the breeding or wintering grounds, or during migration. Because stored fat is the major fuel source for migratory songbirds (Blem 1990), the level of fat stores may have survival consequences for birds during their migration and after arrival on their breeding or wintering grounds (Cherry 1982, Sandberg and Moore 1996). Kuenzel and Helms (1974) reported that WS males had lower fat class scores than TS males during pre-basic molt. I investigated whether different plumage and sex classes of White-throated Sparrows carried different levels of fat at Braddock Bay, on the southern shore of Lake Ontario, during fall and spring migrations. If the plumage and sex classes do indeed carry different levels of fat, this may affect survival during migration, or on the wintering or breeding grounds. I determined a lipid index for each bird by using the deuterium dilution technique to measure fat stores. The deuterium dilution technique accurately and precisely estimates fat mass in White-throated Sparrows (Whitman 2002) and other passerines (Karasov and Pinshow 1998), while the lipid index (g fat/g fat-free dry mass) attempts to account for the amount of fat carried by a bird relative to its body size (Odum et al. 1964, Odum et al. 1965).

Additionally, I examined the plumage morph frequency for each sex during fall and spring migrations to determine if they did indeed differ from a 1:1 ratio. I also compared these data to within sex plumage morph frequencies from previous published studies and past banding results from Braddock Bay, NY.

METHODS

FIELD AND COLLECTION

All White-throated Sparrows were captured as part of regular banding operations conducted by Braddock Bay Bird Observatory (BBBO) in Greece, NY (43°19′24″N, 77°43′03″W). Birds were captured during fall (27 September – 11 October) 2003 and spring (23 April – 12 May) 2004. BBBO is located on the south shore of Lake Ontario in New York State; the habitat includes a mixture of grassland, shrubland, wetland, deciduous forest, and residential areas. BBBO personnel trapped the birds using mist-nets, banded them, and measured un-flattened wing chord lengths. Upon completion of their banding and measurement procedures, BBBO personnel handed captured White-throated Sparrows over to me. I then took digital pictures of each bird. I determined the plumage morph of birds captured in spring by visual inspection. Because plumage morph of White-throated Sparrows in basic plumage cannot be determined by visual inspection, I determined plumage morph of fall birds using the following formula (Piper and Wiley 1989), which they found determined the plumage morph of White-throated Sparrows with 89% accuracy:

Eq. A. $X = (M + L) / (A + S)$

Where M = score of median crown stripe, L = score of lateral crown stripe, A = age score (HY = 1, AHY = 2), and S = sex score (female = 1, male = 2). Birds with $X > 2.0$ were WS, while those with $X < 2.0$ were TS. Birds with $X = 2.0$ were not assigned a plumage morph.

I gave each bird an intraperitoneal injection of 200-300 µl 99 atom % excess ^2H_2O (Isotec, Miamisburg, OH). After the injection, I held all birds in separate cloth

bags for 1 hr prior to drawing blood, so that ^2H_2O equilibrated with body water (Whitman 2002). I took an enriched blood sample from each bird's brachial vein using a 27 gauge needle, and collected the blood using 1-2 heparinized micro-hematocrit capillary tubes. After collection, all blood samples were transferred to 1.5 ml Eppendorf tubes and kept on ice for storage and transport. Before release, I weighed each bird on a digital balance (± 0.1 g). Immediately upon return to the lab, I spun the blood samples in a micro-centrifuge, and then transferred the plasma portions to separate 1.5 ml Eppendorf tubes. Both plasma and red blood cell portions were stored at –20°C for later analysis.

DEUTERIUM DILUTION

I determined the deuterium enrichment of each sample by analyzing it on a gas-chromatography mass-spectrometer (GC-MS). Since plasma water cannot be run directly on a GC-MS, I followed a modified version of the acetone exchange technique developed by Yang et al. (1998). For details of this modified acetone exchange technique, please refer to McCabe et al. (2006).

I calculated total body fat using the following equation developed for White-throated Sparrows in a validation study by Whitman (2002):

Eq. 1. fat mass (g) = total body mass (g) – ^2H dilution space (ml) – (^2H dilution space (ml) / 2.885).

To calculate ^2H dilution space, I employed the following equations (McCabe et al. 2006):

Eq. 2. ^2H dilution space (ml) = ^2H_2O injected (ml) / absolute ^2H enrichment of plasma

Eq. 3. absolute ^2H enrichment of plasma = [m/z 59 / (m/z 58 + m/z 59) – b] / m

Where m/z 59 is the mass spectrometry (MS) signal for ^{2}H labeled acetone and m/z 58 is the MS signal for unlabelled acetone, b = y-intercept of the standard curve, and m = the slope of the standard curve. Terms b and m are used to correct the plasma water ^{2}H enrichment against the standard curve of natural ^{2}H enrichment of water.

After calculating total body fat, I determined a lipid index for each bird to account for the amount of fat carried relative to body size (Odum et al. 1964, Odum et al. 1965, Owen and Cook 1977).

Eq. 4. lipid index = fat mass / fat-free dry mass

In order to calculate fat-free dry mass I used the following simple subtraction:

Eq. 5. fat-free dry mass = total body mass – (water mass + fat mass)

I used the following equation based upon Child and Marshall (1970) to calculate water mass:

Eq. 6. water mass = fat-free mass * 0.6785

Where 0.6785 is the combined mean water ratio for White-throated Sparrows in sub-groups A and B as determined by Child and Marshall (1970).

MOLECULAR SEXING

Following the molecular methods of Griffiths et al. (1998), I determined the sex of each bird. However, I employed direct polymerase chain reaction (PCR) techniques using a modified version of Bercovich et al. (1999) and Tomasulo et al. (2002). I substituted a 1% suspension of red blood cells from the pellet for diluted whole blood. Following the manufacturer's (Takara Inc.) recommendations, I prepared the PCR reactions using 5 ml of 1% pellet suspension in a 25 ml final reaction volume. As per Bercovich et al. (1999), I added 0.625 units of Taq polymerase after the initial

denaturation cycle. Using a Bio-Rad Gene Cycler™ thermal cycler, I amplified the DNA fragments. Using gel electrophoresis, I then ran the PCR products through a 3% agarose gel for at least 1 hr to separate the CHD-W and CHD-Z bands. One band indicated a male, while two bands indicated a female (Griffiths et al. 1998).

STATISTICAL ANALYSIS

Within each season, I tested for proportional differences between plumage morphs for each sex using χ^2 goodness-of-fit tests, assuming expected distributions of 1:1. To detect between season differences for the plumage classes of each sex, I used χ^2 tests. For a separate analysis, I also examined plumage morph frequencies for each sex from BBBO spring banding data collected between 1992 and 1999. BBBO did not record plumage morph data for fall migration because plumage morph of White-throated Sparrows is not easily determined in basic plumage. For birds with plumage morph identified, I used wing chord to determine sex (males $\geq$ 74 mm, females $\leq$ 67 mm) as prescribed by the United States Fish and Wildlife Service [USFWS] and Canadian Wildlife Service [CWS] (1980, Chapter 2). To check for proportional differences between plumage morphs for each sex for BBBO spring banding data between 1992 and 1999, I used χ^2 goodness-of-fit tests. I also used wing chord to determine sex for spring 2004 migrants. To detect differences between the plumage classes of each sex determined by molecular sexing and wing chord sexing methods, I used Fisher exact tests because not all groups had $n > 5$. I employed a general linear model to examine the effects of plumage, sex, and the interaction of plumage and sex on lipid index within each season. To test for differences in lipid index and mass between sexes, and wing chord length between plumage and sex classes, I used two-sample t-tests. Since lipid index was

normally distributed for each sex during both seasons, I followed Blem (1984) and did not transform these data. I used the Minitab statistical program version 14.12.0 (Minitab 2004) to perform all statistical tests according to Zar (1999). All tests were two-tailed and considered significant at the 0.05 level of significance; values reported are means ± SE unless otherwise stated.

RESULTS

I determined the plumage morph and sex of 72 fall migrant and 85 spring migrant White-throated Sparrows. During fall migration, I processed significantly more TS females than WS females ($\chi^2_1 = 7.5$, $P = 0.006$, Table 4.1). Within sex, plumage classes did not differ significantly from a 1:1 ratio for fall males ($\chi^2_1 = 0.9$, $P = 0.330$) or spring males ($\chi^2_1 = 0.4$, $P = 0.505$) and females ($\chi^2_1 = 1.0$, $P = 0.317$, Table 4.1). Within sex, proportions of each plumage class were not significantly different between seasons (males: $\chi^2_1 = 1.3$, $P = 0.247$; females: $\chi^2_1 = 2.3$, $P = 0.126$, Table 4.1). Using only wing chord criteria to sex spring migrants, I found proportionately more TS than WS females ($\chi^2_1 = 4.8$, $P = 0.029$), but not more WS than TS males ($\chi^2_1 = 2.6$, $P = 0.109$, Table 4.1). However, for spring migrants, within sex proportions of each plumage class did not differ significantly between molecular and wing chord sexing techniques (males: $P = 0.353$, Fisher exact test, females: $P = 0.246$, Fisher exact test).

For BBBO spring banding data collected between 1992 and 1999, I found 121 White-throated Sparrows with plumage morph identified and sexable by wing chord. For females, there were significantly more TS ($n = 54$) than WS ($n = 6$) birds ($\chi^2_1 = 38.4$, $P <$

0.001, Table 4.2). There were also significantly more WS (n = 39) than TS (n = 22) males ($\chi^2_1 = 4.7$, $P = 0.030$, Table 4.2).

The interaction between plumage and sex had a significant effect on lipid index of fall migrant White-throated Sparrows (Table 4.3, Fig. 4.1). Although neither plumage nor sex alone significantly affected lipid index, the interaction between sex and plumage was significant (Table 4.3). During fall migration, TS females had lower lipid index values than other plumage and sex classes (Fig. 4.1). There was no significant influence of plumage, sex, or the interaction between plumage and sex, on lipid index of spring migrants (Table 4.4, Fig. 4.1).

Lipid index did not differ significantly between male and female migrants during fall ($t_{59} = 1.7$, $P = 0.095$, Table 4.5) or spring ($t_{81} = 1.0$, $P = 0.312$, Table 4.5), although males were heavier than females in both fall ($t_{59} = 7.4$, $P < 0.001$) and spring ($t_{81} = 6.0$, $P < 0.001$, Table 4.6).

There was no mass difference between WS and TS males during fall ($t_{29} = 0.5$, $P = 0.640$) or spring ($t_{34} = 1.1$, $P = 0.920$, Table 4.6), or between the mass of WS and TS females during fall ($t_{27} = 1.2$, $P = 0.252$) or spring ($t_{46} = 1.1$, $P = 0.296$, Table 4.6). However, WS males (73.3 ± 0.3 mm) had longer wing chord lengths than TS males (72.3 ± 0.3 mm) ($t_{72} = 2.3$, $P = 0.024$, Fig. 4.2), and WS females (69.1 ± 0.3 mm) had longer wing chord lengths than TS females (67.9 ± 0.2 mm) ($t_{81} = 3.4$, $P = 0.001$, Fig. 4.3).

DISCUSSION

On the breeding grounds, WS male x TS female pairs (57% - 79%) are more common than TS male x WS female pairs (14% - 41%) (Lowther 1961, Lowther and Falls 1968,

Thorneycroft 1975, Knapton and Falls 1982, Tuttle 1993). Additionally, adult WS males (63% - 68%) outnumber TS males (32% - 37%), and TS females (74% - 78%) outnumber WS females (22% - 26%), by similar degrees (Lowther 1961, Thorneycroft 1975). However, plumage morph frequencies of young birds (eggs, nestlings, fledglings, and juveniles) do not differ for either sex (males: WS = 57%, TS = 43%; females: WS = 50%, TS = 50%) (Thorneycroft 1975). These data suggest that differential mortality between plumage and sex classes of first year birds occurs somewhere between the time that birds fledge and when they arrive on the breeding grounds the following spring (Falls and Kopachena 1994). It is unclear if consistent differential mortality occurs between plumage and sex classes of adult birds, as adult tan-striped birds of both sexes showed higher return rates than white-striped birds in Adirondack Park, New York (Tuttle 1993), but not at Algonquin Park, Ontario (Knapton et al. 1984).

During the course of this study, I found significantly more TS females than WS females during fall migration. However, male plumage class frequencies did not differ significantly from a 1:1 ratio during fall migration. Thorneycroft (1975) detected no plumage morph frequency differences for male and female migrants, although he lumped fall and spring migrants. However, using wing chord criteria to assign sex (females ≤ 69 mm, males ≥ 74 mm), researchers at Powdermill Avian Research Center, Rector, Pennsylvania, reported more WS (59%) than TS (41%) males and more TS (99%) than WS (1%) females (Leppold personal communication).

On White-throated Sparrow wintering grounds, plumage morph frequencies for each sex are intermediate between young (Thorneycroft 1975) and adult (Lowther 1961, Thorneycroft 1975, Watt et al. 1984, Piper and Wiley 1989) birds on the breeding

grounds suggest that some differential mortality occurs during fall migration or the winter. However, Piper and Wiley (1989) observed no difference in plumage morph frequencies for wintering females. The dissimilar degrees of plumage morph frequency differences for each sex between winter and summer suggest that some differential mortality also occurs during spring migration.

During spring migration, plumage class frequencies of both sexes did not differ significantly from a 1:1 ratio in this study. Thorneycroft (1975) also detected no plumage morph frequency differences for migrants of both sexes, although he lumped fall and spring migrants. In contrast, spring banding data from BBBO for 1992 to 1999 showed significantly more WS (64%) than TS (36%) males, and more TS (90%) than WS (10%) females. Data for males appear to conform to the 2:1 breeding ground ratio, while data for females greatly exceeds the 2:1 breeding ground ratio. Falls and Kopachena (1994) reported that at Long Point, Ontario, Houtman (unpublished data) observed plumage morph frequencies for spring migrants of both sexes that were similar to the 2:1 breeding ground ratio. Additionally, using wing chord criteria to assign sex (females $\leq$ 69 mm, males $\geq$ 74 mm), researchers at Powdermill Avian Research Center, Rector, Pennsylvania, reported more WS (75%) than TS (25%) males and more TS (76%) than WS (24%) females (Leppold personal communication).

In agreement with previous reports (Odum and Perkinson 1951, Wolfson 1954, Kuenzel and Helms 1974), I found that male White-throated Sparrows were significantly heavier than female White-throated Sparrows. However, males did not have higher lipid index values and therefore did not carry relatively more fat than females during either migration. Except during the pre-basic molt period when TS males averaged about 0.5

fat class higher than WS males, Kuenzel and Helms (1974) found no difference in fat class for all plumage and sex classes. Additionally, both lipid index (Table 2.1, Fig. 4.1) and fat class (Kuenzel and Helms 1974, Table 2.1) were higher during spring migration for all plumage and sex classes, as was the case in my study.

The interaction between plumage and sex had a significant effect on lipid index of fall migrant, but not spring migrant, White-throated Sparrows. With no difference in lipid index between plumage classes for either sex of spring migrants, it is unlikely that fat stores influenced any differential mortality between plumage and sex classes that may have occurred during spring migration. Instead, factors other than plumage and sex class (age, dominance status, location, etc.) probably accounted for differences in fat stores among individuals. Interestingly, during fall migration only TS females had lower lipid index values than other plumage and sex classes. However, I captured significantly more TS females than WS females during fall migration. This suggests that higher lipid index values, and therefore higher fat stores, are not related to higher plumage morph frequency of fall female migrants, and that fat stores do not affect survival during fall migration. TS females may have lower lipid index values than other plumage and sex classes during fall migration due to greater time and energy demands during the breeding season (Knapton and Falls 1983). However, during the pre-migratory phase, mass (2.3 – 2.6% per day) and fat stores increase rapidly in other *Zonotrichia* (King et al. 1965, Morton 2002). Fat deposition accounts for most of this rapidly increasing mass (Odum et al. 1964). With at least one month after completion of the breeding season and the start of fall migration (Falls and Kopachena 1994), TS females should have adequate time to acquire fat stores prior to fall migration.

Small sample sizes for fall 2003 and spring 2004 may have limited my ability to detect differences in plumage morph frequencies for both sexes. However, spring plumage morph frequency differences from 1992-1999 may be an artifact of using wing chord to sex White-throated Sparrows. For the spring 1992-1999 data set, I classified those White-throated Sparrows with the largest wing chord lengths as males, and those with the smallest wing chord lengths as females (USFWS and CWS 1980, Chapter 3). However, WS birds have larger wing chord lengths than TS birds. Thus the larger WS males and smaller TS females will tend to be overrepresented in the sample. This was indeed the case, as I found significantly more TS than WS females and nearly significantly more WS than TS males when I used wing chord criteria to sex the spring 2004 migrants. Additionally, sexing White-throated Sparrows by wing chord leaves a large proportion of birds unidentified (Chapter 3). Thus, any plumage and sex class frequencies that use wing chord to determine sex should be considered suspect. Studies that used wing chord to sex White-throated Sparrows include Watt et al. (1984), Houtman (unpublished), Leppold (unpublished) and the BBBO 1992-1999 data set. For studies of plumage class frequencies of White-throated Sparrow populations, molecular sexing or laparotomy should be used instead.

During fall and spring migrations, most plumage and sex class frequency data based on molecular sexing (this study) and laparotomy (Thorneycroft 1975) do not support differential mortality between the plumage and sex classes. While I did observe significantly more TS females in the fall, I did not observe more TS females in the spring, based on molecular sexing. However, my sample sizes were relatively small and this may have obscured the results. Future studies of plumage and sex class frequencies with

larger sample sizes should help alleviate this problem. On North Carolina wintering grounds, Piper and Wiley (1989) detected more WS than TS males, but observed no difference between WS and TS females. However, their results may not be applicable across the entire winter range of the species, due to differential migration of the sexes (Jenkins and Cristol 2002), and possibly plumage morphs. Jenkins and Cristol (2002) reported that females tend to winter further south than males. However, Jenkins and Cristol (2002) used wing chord to determine sex, with smaller birds classified as females and larger birds as males. They did not report any information on plumage morph, but because TS birds are smaller than WS birds, it is possible that the smaller TS birds winter further south than WS birds. If this is the case, then TS birds, and females in particular should winter further south, and males and WS birds should winter further north. Differential migration by sex and plumage morph may contribute to differential mortality between plumage and sex classes or conceal a lack of frequency differences between the plumage classes, within each sex. More data on plumage and sex class frequencies during migration are needed to help determine whether the observed frequency discrepancy in breeding pairs is due to differential mortality or is merely an apparent difference.

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TABLE 4.1. Percent of each plumage morph by sex for fall 2003 and spring 2004 migrant White-throated Sparrows processed at Braddock Bay Bird Observatory, Greece, NY. Sample size is shown in parentheses.

	Males		Females	
	White-striped	Tan-striped	White-striped	Tan-striped
Fall[1]	42% (16)	58% (22)	26% (9)	74% (25)
Spring[1]	56% (20)	44% (16)	43% (21)	57% (28)
Spring[2]	71% (10)	29% (4)	19% (3)	81% (13)

[1] Birds sexed with molecular methods.

[2] Birds sexed by wing chord (USFWS and CWS 1980).

TABLE 4.2. Percent of each plumage morph by sex for spring migrant White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, between 1992 and 1999. Sample size is shown in parentheses.

Males		Females	
White-striped	Tan-striped	White-striped	Tan-striped
64% (39)	36% (22)	10% (6)	90% (54)

TABLE 4.3. General Linear Model for effects of plumage morph, sex and the interaction between plumage morph and sex on lipid index of fall migrant White-throated Sparrows.

Source	df	Seq. SS	Adj. SS	Adj. MS	F	P
Sex	1	0.1103	0.0084	0.0084	0.24	0.626
Plumage	1	0.0395	0.0730	0.0730	2.10	0.153
Sex * Plumage	1	0.2106	0.2106	0.2106	6.06	0.017
Error	56	1.9464	1.9464	0.0348		
Total	59	2.3068				

TABLE 4.4. General Linear Model for effects of plumage morph, sex and the interaction between plumage morph and sex on lipid index of spring migrant White-throated Sparrows.

Source	df	Seq. SS	Adj. SS	Adj. MS	F	P
Sex	1	0.0908	0.1016	0.1016	1.10	0.297
Plumage	1	0.0284	0.0305	0.0305	0.33	0.566
Sex * Plumage	1	0.0030	0.0030	0.0030	0.03	0.856
Error	79	7.2683	7.2683	0.0920		
Total	82	7.3904				

TABLE 4.5. Mean mass and lipid index [fat mass (g) / fat-free dry mass (g)] for fall and spring migrant White-throated Sparrows processed at Braddock Bay Bird Observatory, Greece, NY. All mean values are ± SE.

	Fall			Spring		
	Mass	Lipid Index	*n*	Mass	Lipid Index	*n*
Males	25.3 ± 0.2	0.50 ± 0.03	31	27.2 ± 0.3	1.10 ± 0.05	48
Females	23.0 ± 0.2	0.41 ± 0.04	30	24.7 ± 0.3	1.03 ± 0.05	35

TABLE 4.6. Mean mass for fall and spring migrant White-throated Sparrows processed at Braddock Bay Bird Observatory, Greece, NY. WS = white-striped, TS = tan-striped. All mean values are ± SE.

	Fall				Spring			
	WS	*n*	TS	*n*	WS	*n*	TS	*n*
Males	25.5 ± 0.3	12	25.3 ± 0.3	19	27.3 ± 0.4	20	27.2 ± 0.5	20
Females	23.4 ± 0.6	7	22.8 ± 0.2	22	25.0 ± 0.4	16	24.4 ± 0.4	28

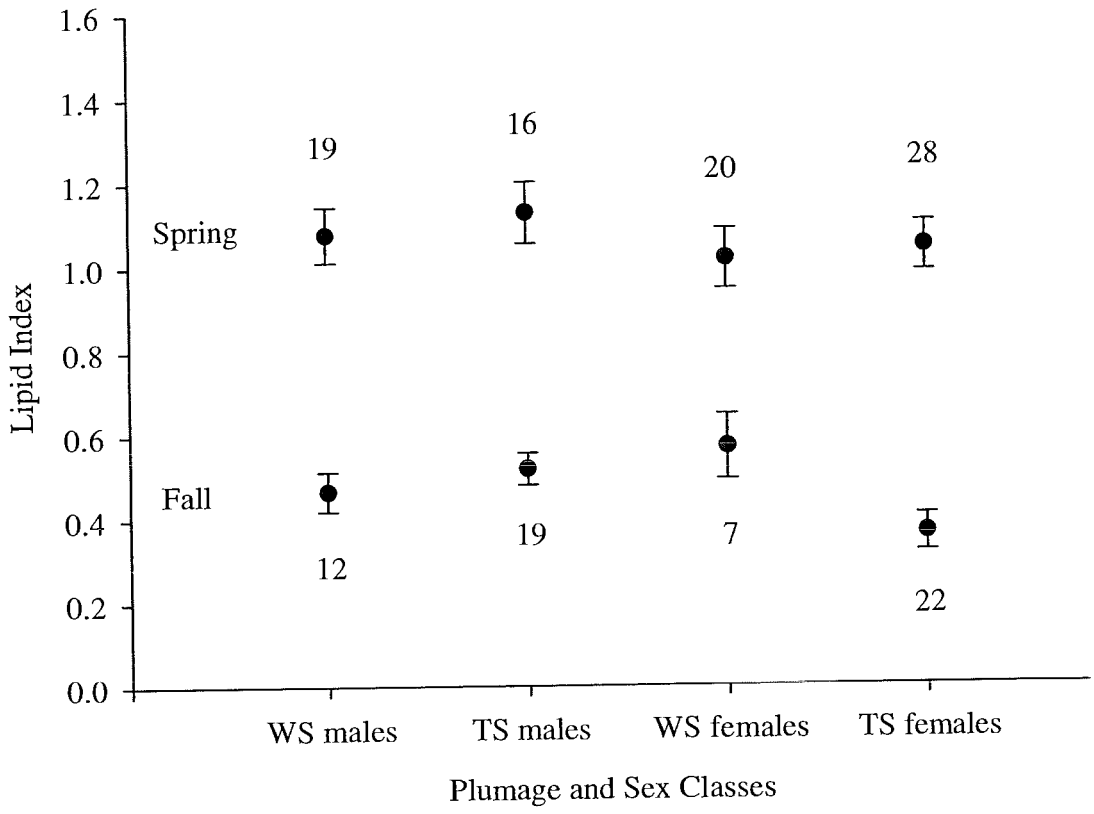

FIGURE 4.1. Mean lipid index [fat mass (g) / fat-free dry mass (g)] ± SE for plumage and sex classes of fall and spring migrant White-throated Sparrows processed at Braddock Bay Bird Observatory, Greece, NY. WS = white-striped, TS = tan-striped. Samples size is shown below fall and above spring plots.

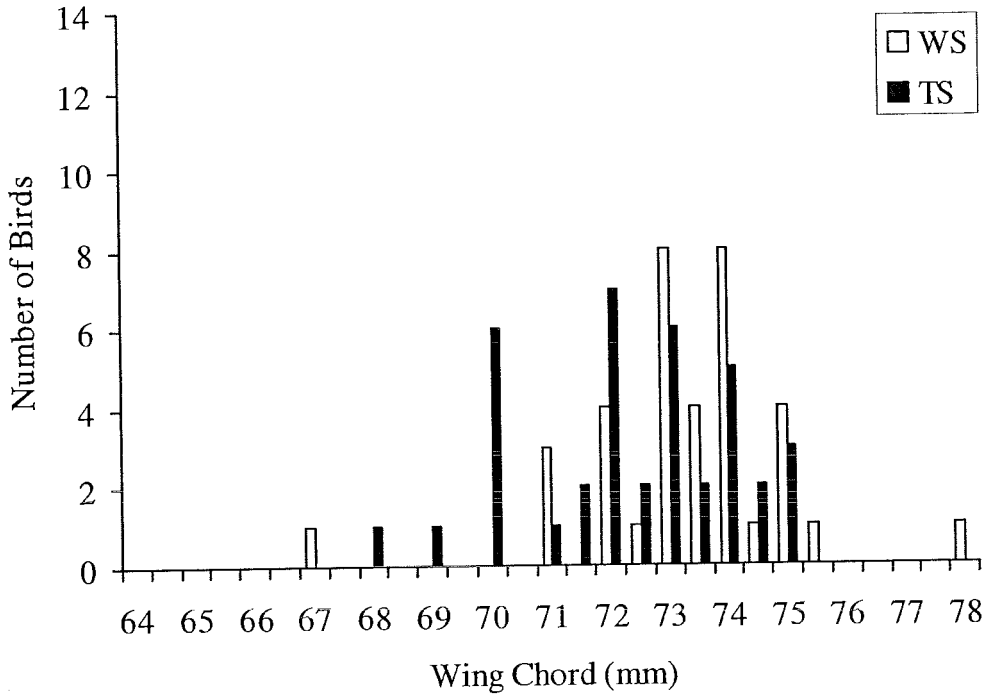

FIGURE 4.2. Distribution of wing chord measurements for white-striped (WS, $n = 36$) and tan-striped (TS, $n = 38$) male White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during Fall 2003 and Spring 2004.

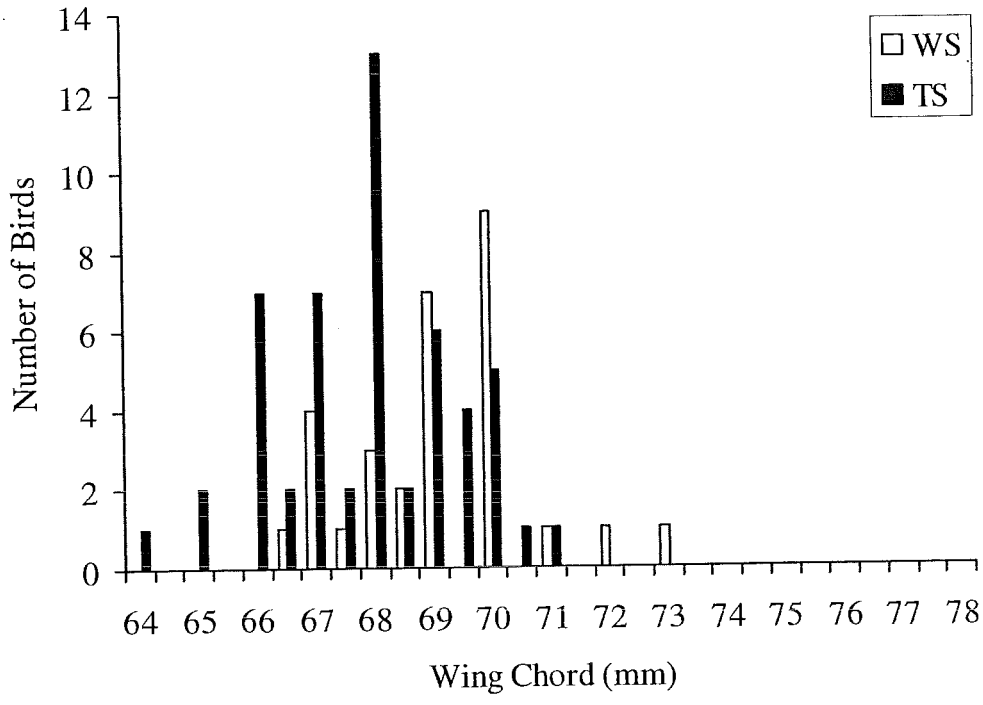

FIGURE 4.3. Distribution of wing chord measurements for white-striped (WS, $n = 30$) and tan-striped (TS, $n = 53$) female White-throated Sparrows captured at Braddock Bay Bird Observatory, Greece, NY, during Fall 2003 and Spring 2004.

CHAPTER FIVE

Conclusions

CONCLUSIONS

The main objective of my study was to measure lipid stores in White-throated Sparrows of different plumage and sex classes during fall and spring migrations. In turn, I hoped that these data would help elucidate mechanisms at least partially responsible for maintaining the greater proportion of WS than TS males and TS than WS females observed on the breeding grounds.

Using the deuterium dilution technique to measure lipid index, I found that TS females had a lower lipid index than other plumage and sex classes during fall migration. Additionally, the interaction between plumage and sex significantly affected lipid index during fall migration. Yet, TS females were more common than WS females during fall. Lower fat stores were not correlated with lower plumage morph frequency. It is unlikely that greater time and energy demands during the breeding season (Knapton and Falls 1983) are responsible for lower lipid index values of TS females during fall migration. During spring migration, there was no difference in lipid index between plumage morphs of either sex, and the interaction between plumage and sex did not significantly affect lipid index. It does not appear that fat stores influence survival (as determined by relative frequency in the population) of plumage morphs for either sex, during either fall or spring migrations.

Differences in lipid index between adjacent fat classes were not linear, which supports Hailman's (1965, 1969) contention that fat class data are of an ordinal and not an interval scale. Additionally, there was a significant interaction between fat class and season on lipid index. Lipid index for a given fat class in spring was significantly higher than lipid index for the same fat class in fall. Furthermore, the rate of lipid index increase

between fat class levels during spring was twice the rate for the fall. Fat classes should not be compared between seasons, both because they represent different amounts of fat stores in different seasons, and because fat stores increase at different rates between adjacent fat classes in different seasons. Instead, a quantitative method of measuring fat content such as the deuterium dilution technique should be used. Additionally, assigning fat classes should not be used to study birds with low fat stores, since birds with a fat class of 0 still registered lipid index values well above birds know to have depleted their fat reserves (Rogers 1991). However, assigning fat classes is still a reasonable method to estimate relative fat stores within seasons.

Determining sex of White-throated Sparrows was best accomplished by molecular techniques (Griffiths et al. 1998). While males do tend to have longer wing chord lengths than females, the large degree of wing chord length overlap between the sexes makes it impossible to identify sex for many birds. Wing chord length can still be used to determine sex of some White-throated Sparrows, but caution should be exercised when deciding on which criteria to use. Choosing more inclusive criteria (Pyle 1997) allows one to assign sex for more birds, but also leads to some cases of mis-identification. In order to eliminate mis-identifications, more conservative criteria (USFWS and CWS 1980) should be used, but then fewer birds can be sexed. Ideally, molecular sexing techniques should be used to determine sex of White-throated Sparrows or other passerines. In the field, spending a couple minutes to take a small blood sample is all that is required. And given access to molecular biology equipment, lab work for these techniques is neither difficult nor expensive.

WS birds also have longer wing chord lengths than TS birds. Even though there is greater overlap in wing chord length between the plumage morphs than for the sexes, using wing chord length to sex White-throated Sparrows will have a confounding effect on within sex plumage morph frequencies. WS males and TS females will tend to be over-represented in populations sexed by wing chord. Therefore, within sex plumage morph frequencies reported by studies that use wing chord to determine sex of White-throated Sparrows should be interpreted with caution.

During fall migration, TS females significantly outnumbered WS females. However, male plumage morph frequencies did not differ from a 1:1 ratio in fall. During spring migration, plumage morph frequencies for each sex did not differ from a 1:1 ratio. Overall, these plumage class frequency data do not support differential mortality between plumage classes for either sex during the non-breeding season, especially since plumage morph frequencies for both sexes were not different from a 1:1 ratio during spring migration. However, these data are in accord with the hypothesis that the skewed plumage morph frequencies for each sex observed on the breeding and wintering grounds are due to apparent and not actual differences in plumage morph frequencies. Thus, differential mortality may not be responsible for why WS males outnumber TS females and TS females outnumber WS females on the breeding and wintering grounds. Instead the observed differences may be apparent. Differential migration (Jenkins and Cristol 2002), habitat preferences (Knapton and Falls 1982), neighbor distance preferences (Formica et al. 2004), and/or detection issues (Lowther 1961, Lowther and Falls 1968, Knapton et al. 1984, Tuttle 1993) may all be involved with the observed differences in plumage class frequencies seen for each sex.

Future studies should employ molecular sexing to monitor within sex plumage morph frequencies during spring migration at several locations across North America. Development of a molecular method to replace karyotyping as the definitive means to identify plumage morph of White-throated Sparrows would simplify accurate and reliable plumage morph identification. Additionally, molecular plumage morph identification would greatly improve upon the accuracy (89%) of Piper and Wiley's (1989) technique to identify plumage morph of White-throated Sparrows during fall or winter, and as fledglings or nestlings. Any study that focuses on within sex plumage morph frequencies of White-throated Sparrows would greatly benefit from the ability to accurately and reliably identify both sex and plumage morph from a single blood sample. Future studies could employ both molecular sexing and molecular plumage morph identification to measure within sex plumage morph frequencies during fall migration and winter at various locations across North America.

Since any differential mortality that may occur probably happens among first year birds, I recommend detailed studies of first year White-throated Sparrow survival as well as evidence of differential migration by age class. For instance, molecular sexing and molecular plumage morph identification could be used to measure within sex plumage morph frequencies of populations of first year birds at different developmental stages, times of year (nestling, fledgling, end of summer, fall migration, winter, spring migration), and geographic locations. Such a detailed study would identify when, where or if differential mortality occurs among first year birds.

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